

Fishing for tilapia sex genes by Chr3 micro-dissection

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Sex control is needed in tilapia production to overcome female continuous reproduction and to benefit from males fast growth-rate. Genetic control in this group requires an understanding of the complex sex determination as well as the finding of the sex-determining gene(s). Within the tilapia group, the sex determining locus has been located on linkage group 1 (LG1) in *Oreochromis niloticus*, whereas in *O. aureus* LG3 or both LG1 and LG3 are sex-linked depending upon the populations and strains. Using specific BAC clones as probes for FISH, LG3 and LG1 have been respectively located on the largest and on a smaller chromosome pairs. The largest pair presents various traits of a relatively old sex chromosome, whereas LG1 seems to be at an early stage of sex chromosome evolution. Evidence for interactions between the two sex linked loci have been demonstrated at least in *O. aureus*, with minor factors (genetic and environmental factors) also modulating sex ratios. We are interested in identifying and isolating genes present in the tilapia sex chromosomes in order to obtain valid sex-linked markers to be used for fish farming. While waiting for the tilapia genome sequencing, these sex markers will help physical mapping of the two linkage groups LG1 and LG3 which have been anchored to the two sex chromosomes, and better refine the region of the sex determining gene(s). In the present study we have taken advantage of the large size of the "old chromosome" containing LG3, hereafter called Chr3, to micro-dissect it in order to search for genes present in this chromosome. The micro-dissection of Chr3 was performed from metaphase spreads prepared from female XX and male YY genotypes in the Nile tilapia *O. niloticus* while male ZZ were used from *O. aureus*. The micro-dissection of chromosomes was coupled to the procedures of cDNA capture and direct cDNA selection. We obtained several transposons but a reduced number of genes. This may have been due to a DNA bias perhaps

preferentially amplifying repetitive elements, when amplifying the chromosomes after dissection with degenerate oligonucleotide primer PCR (DOP-PCR). It is a widely used whole genomic amplification method (WGA) frequently used for chromosome painting preparations and comparative genomic hybridizations (CGH). Three different WGA methods (DOP-PCR, GenomePlex and GenomiPhi) were subsequently compared on a pool of 30 micro-dissected chromosomes to evaluate which affected the least the Chr3 genome representation. Loci consisting in five microsatellites, two genes and two uncoded fragments all located on Chr3 were then searched by PCR on the DNA obtained from the three WGAs. Chr3 representation varied with the WGA method used but both GenomePlex and GenomiPhi gave 60% loci amplification. When the DNAs were used as chromosome painting probes, only GenomePlex gave good signals which were mainly located on the Chr3 pair and covering it nearly entirely. In the centromeric regions light signals were observed with differences seen between XX and YY. Differences were also observed between the sexual genotypes towards the telomeric regions, which correspond to the putative location of the female sex-determinant of *O. aureus*.

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